Influence of the Environment on the Unborn

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• **Embryonic development is influenced by three environments: the intra-uterine, the maternal body and the maternal surroundings. Factors present in one or other may cause abnormal development.**

Usually environmental factors act in association with genetic factors but they can be the dominant or sole cause of birth defects.

Many malformation-producing agents exist in the maternal environment and some cause abnormalities in man. Use of such agents in experimental animals yields valuable information on how malformations develop.

Different species and strains of animals often react differently to the same teratogenic agent, leading to difficulties in screening substances which may be harmful to human embryos.

Laboratory experimentation, detailed study of human abortion material and the vigilance of the physician are all essential in the search for human teratogens.

THROUGHOUT OUR LIVES we are constantly reacting to the environment in which we live. Heat, light, atmospheric pressure, terrestial and extraterrestial radiation, gravity, microorganisms and the multitude of chemicals contained in food, water and air are continually acting upon us, determining our constitutions and our destinies. At one time it was felt that the mammalian fetus was relatively sheltered from the effects of such environmental factors but careful clinical and experimental studies have now shown this belief to be untenable.

While the mother does afford protection to the unborn in many ways—for example, by detoxifying noxious substances and by destroying microorganisms which would be harmful were they to reach the young—this is secondary to the preservation of her own organism. Where the agent is not harmful to the mother and protective reactions are absent, the effect on the embryo can be disastrous. Indeed, the majority of teratogenic (malformation-producing) agents or procedures belong to this category and are especially destructive in the early stages of gestation. Thus, rubella, if contracted by the mother during the first trimester, causes little maternal upset but may result in serious eye, ear and cardiovascular malformations in the embryo.[11] Again, maternal ingestion of thalidomide, a glutamic acid imide that once was supposed to be a harmless sedative, has recently been linked with a syndrome of phocomelia, cavernous angioma and duodenal stenosis in the offspring.[17]

The Maternal and Embryonic Environments

The unborn has to contend with three environments. The one with which it is in immediate contact, consisting of the amniotic fluid, the placenta and membranes, has been designated the *microenvironment* by Warkany.[7] The maternal body may be called the *macroenvironment*, and the surroundings of the mother, the *matronenvironment* (Figure 1). Substances inhaled or ingested by the mother from the matroenvironment may reach the embryo unchanged, or may produce changes in the macroenvironment or microenvironment which are

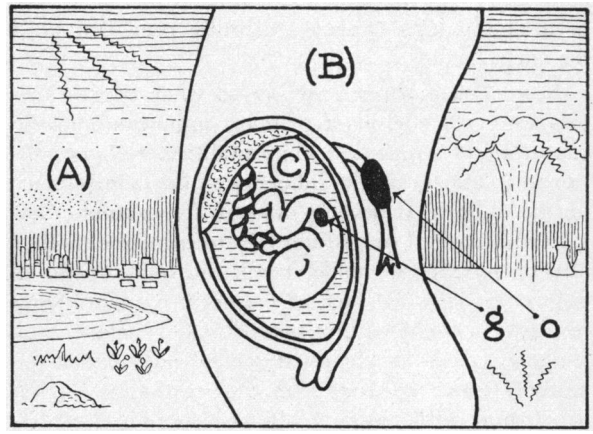

Figure 1.—The maternal and embryonic environments. (A) the *matroenvironment* consisting of the physical and chemical components, and the animal and plant life, in the surroundings of the mother. Radiation from outer space, the earth, and man-made sources is indicated by wavy lines; (B) the *macroenvironment* or maternal body; (C) the *microenvironment* composed of the placenta, membranes and amniotic fluid. (B) and (C) constitute the embryonic environment.

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TABLE 1.—*Some Factors Which Produce Malformations*

	In Animals	In Man
Physical	Radiation, hypothermia	Radiation
Chemical:		
Hormones	Insulin, cortisone, androgen, estrogen, epinephrine	Sex hormones
Antigrowth factors	Nitrogen mustard, chlorambucil, azaserine, 6-mercaptopurine	?
Other	Trypan blue, quinine, hypoxia, salicylate, colchicine, iodine deficiency, antibiotics	Thalidomide
Nutritional:		
Deficiency	Vitamins A, B_{12}, D, E,* folic acid (PGA*), pantothenic acid,* nicotinic acid,* riboflavin*	Aminopterin†
Excess	Vitamin A	.....
Other	Starvation	.....
Micro-organismal	Hog cholera, influenza A, Newcastle virus	Rubella, syphilis, toxoplasmosis

*Vitamin antagonist employed alone or with deficient diet.
†A folic acid antagonist.

ultimately experienced by the embryo. It is also possible for substances to pass from the macroenvironment and accumulate in the microenvironment in large enough amounts to cause embryonic damage. In the case of radiation the embryo may be directly affected, or it may be affected by the products of reaction with the macroenvironment.

Environment and Genetic Factors

Nineteenth century experimental embryologists clearly showed that environmental change could disturb development both in invertebrates and in lower vertebrates, and by the beginning of the present century considerable attention was being directed to abnormal intrauterine conditions as causes of malformation and abortion in man.[2,18] However, the importance of inherited factors in the normal and abnormal development of mammals was now appreciated and the significance of the environment gradually became subordinated to that of the germ-plasm; this concept generally prevailed until the early forties.

Nevertheless, during the period when genetic factors were considered of primary importance in the causation of congenital abnormalities, reports continued to appear on the influence of the environment on the unborn. It became evident, for example, that x-irradiation[13,14] or radium treatment of the mother during pregnancy could result in fetal death or deformity, and that lack of iodine in pregnant sows resulted in reduced litter-size.[27] Cleft palate[3] was frequently seen in whelps of captive lions unless the mothers were fed goat flesh and soft bone during pregnancy, while sows receiving a vitamin A-deficient diet produced piglets with eye defects[12] and other malformations. However, it was not until 1940 and the publication of a study by Warkany and Nelson[28] showing that pregnant rats fed a deficient diet (later shown to be riboflavin deficiency) produced young with skeletal and other abnormalities, that attention was again seriously directed to the influence of the environment on mammalian development. The teratogenic effect of rubella on the human fetus observed soon after this gave further impetus to the renewed interest in environmental factors; since then a host of teratogenic agents or procedures has been discovered (Table 1).

Today, however, it is generally agreed that the majority of congenital abnormalities result from the interplay of *both* genetic and environmental factors[9] although in certain instances one may play a much more important role than the other. Since there is no apparent structural difference between congenital abnormalities produced by genetic and by environmental factors, in many cases it is difficult to determine which is of primary or sole importance.

In addition to malformations resulting from environmental or genetic factors, or to a combination of these, it is now known that abnormality of chromosomal number is responsible for such conditions as Turner's and Klinefelter's syndromes, and for mongolism. What role, if any, environmental or genetic factors play in the determination of such chromosomal disturbance has not yet been determined.

Teratogenic Agents—Timing and Specificity

It would appear that either an excess or an insufficiency of almost any chemical or physical agent can, in certain circumstances, result in defective embryonic development; thus, either maternal insufficiency[31] or excess[5] of vitamin A produces abnormal rat young when occurring at a particular stage of pregnancy.

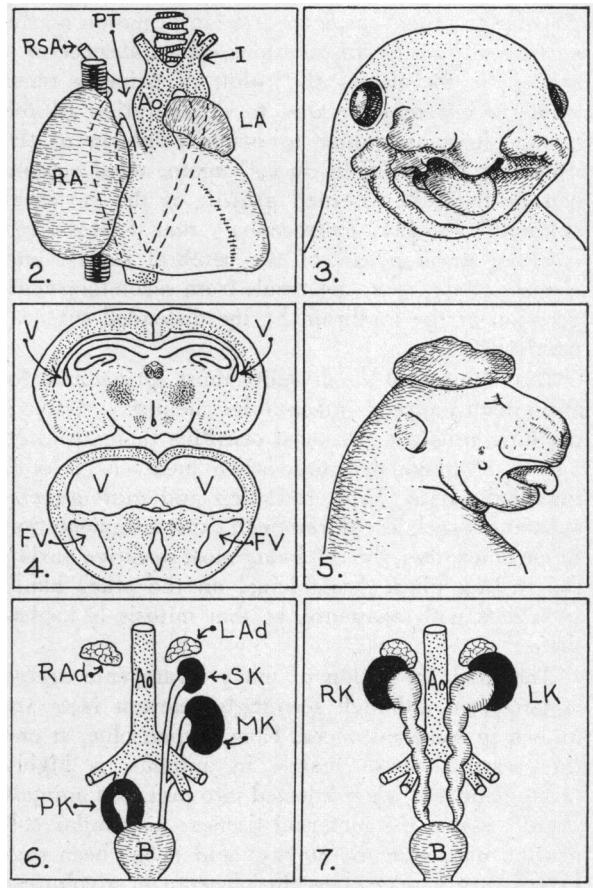
The time of introduction of a teratogenic agent is especially important, the embryo usually being most sensitive when the principal body systems are being established; in man, this is between the third and eighth week, and in rats during the second week of gestation (parturition occurs on the 22nd or 23rd day). In the later stages of pregnancy the fetus is much less sensitive but by no means immune to

environmental influence; thus, in man, toxoplasmosis can produce hydrocephaly, and syphilis a variety of malformations in the later stages of gestation. Also, in rats the giving of 6-aminonicotinamide (6-AN), a nicotinic acid antimetabolite, as late as the 19th day of gestation can produce hydrocephaly in the young.[4]

Generally, when a teratogenic agent is given very early in gestation it either does not disturb the conceptus or it destroys it entirely, while, if given late in pregnancy its effects may be greatly reduced or absent; there is consequently a critical time for each agent during which maximum damage to the conceptus will result, and this varies with the species involved. In the case of thalidomide, for example, it has been observed that the human embryo is most sensitive between the 27th and 43rd days after conception.[17]

While the time of introduction of the agent is undoubtedly of importance, there is generally mounting evidence that certain agents have a predilection for one or more systems or regions of the embryo.[30] This is suggested, for example, by the preponderance of limb damage in human fetuses by thalidomide, and by the frequent absence of the kidney in rat young from chlorambucil.[23] On the other hand, certain teratogenic factors, such as maternal folic or pteroylglutamic acid (PGA) deficiency,[10,25,26] are associated with a broad spectrum of malformations and are considered "universal" teratogens.

The employment of teratogens in experimental animals is exceedingly valuable for studying the pathogenesis of many congenital abnormalities, and different defects can be produced by varying the time of action of the same agent, or by using different agents (Figure 2). Thus, a transitory PGA-deficiency in rats from the 7th to 9th day of gestation produces many abnormalities of the brain and eye; from the 9th to 11th day, mainly cardiovascular abnormalities;[1,21] and from the 10th to 13th days, principally urogenital malformations.[22] However, if it is desired to study dextrocardia or transposition of the great vessels, trypan blue[8] is the agent of choice, as it provides a much higher incidence of these anomalies than maternal PGA-deficiency.



Ao—aorta
B—bladder
FV—false ventricle
I—innominate artery
LA—left atrium
LAd—left adrenal
LK—left kidney
MK—main kidney
PK—pelvic kidney
PT—pulmonary trunk
RA—right atrium
RAd—right adrenal
RK—right kidney
RSA—right subclavian artery
SK—supernumerary kidney
V—lateral ventricle

Figures 2-7.—Congenital abnormalities resembling those occurring in man produced in rat young as a result of trypan blue (Figure 2), and folic acid (PGA) deficiency (Figures 3-7) during pregnancy; (2) Transposition of the great vessels and double aorta; (3 Facial defects and cleft palate; (4) Control and hydrocephalic brains from three-week-old rats; (5) Exencephaly, micrognathia, and glossoptis; (6) Pelvic and supernumerary kidneys; and (7) Bilateral hydronephrosis.

Human Teratogens

Of the great number of agents or procedures recognized as teratogenic in mammals, only a few are definitely known to affect humans. Proven teratogenic agents in man are: rubella, sex hormones, aminopterin (4-amino PGA), toxoplasmosis, radiation and thalidomide (Table 1). Many other chemical substances, physical factors and microorganisms are suspect but absolute proof is lacking.

It is sometimes stated that experimentally produced congenital malformations are caused by dosages of teratogenic agents at levels much greater than ever experienced by man. In many instances this is probably true but the effects of combinations of small amounts of teratogens cannot be overlooked and work on this important aspect is now proceeding.[6] Preliminary results indicate that certain combinations of low dosages of teratogenic agents have an adjuvant effect on the production of malformations while others seem to show a protective effect. The problem, however, is complex and requires more detailed study.

Pathogenesis of Malformations

The ability to produce abnormal embryos in animals by means of teratogenic agents has made it

possible to obtain more accurate information on the genesis of many malformations. Thus, absence of a kidney is not always the result of primary renal agenesis but may be due to degeneration of the metanephros secondary to maldevelopment of the ureter or the Wolffian duct;[23] again, renal ectopia can result from retarded growth of the vertebral column.[22] Further, hydrocephaly may follow from retarded development of the cerebral cortex, and closure of the aqueduct result from secondary compression of the midbrain by the distended cerebral hemispheres.[24]

Any congenital abnormality must spring initially from disturbance of intracellular chemistry. Actively dividing cells are the most sensitive to teratogenic agents, although the phase when such sensitivity is maximal varies. Thus, radiation and radiomimetic substances such as chlorambucil cause fragmentation of chromosomes, the cell being most sensitive during the resting phase; colchicine, on the other hand, interferes with anaphase, so that mitosis is incomplete.

The mode of action of many teratogenic agents is uncertain although seemingly relevant facts are known in some instances. Thus, trypan blue, at one time used to treat mange in animals, is highly teratogenic and when injected into pregnant animals rapidly stains the maternal tissues; no similar coloration occurs in the embryo and it has been suggested that it may cross the placenta in a colorless form. However, when injected into pregnant rabbits, trypan blue alters the serum protein content of the maternal blood[16] and it is possible that this may lead, in turn, to abnormal placental transfer and subsequent fetal abnormality. In the case of PGA-deficiency the formation of nucleoproteins essential for growth and cell-division is probably disturbed; riboflavin deficiency, on the other hand, possibly interferes with oxidative processes in both the mother and the embryo.

The site of primary damage by a teratogenic agent conceivably may be either the placenta or the embryo; studies on the effect of maternal PGA-deficiency, however, have shown that embryonic death precedes placental change, and it is probable that this sequence is common to many teratogenic procedures.[15]

The teratogenic effects of antimetabolites generally can be counteracted by simultaneously supplying an adequate amount of the corresponding vitamin, yet in some instances an entirely different substance may also have an alleviating effect. Thus, in maternal vitamin A-deficiency in rats it has been observed that fetal damage can be reduced by thyroxine.[20] Also, recent studies, again in rats, have shown that thalidomide increases the sensitivity of hemoglobin to oxidation by nitrites and that this can be prevented by simultaneously giving pyridoxine and riboflavin.[19] In the future, it is possible that teratogenic side-effects of otherwise useful drugs may be prevented by prescribing with them antidotes to their undesired effects.

Testing for Teratogenicity

The fact that thalidomide has produced severe malformations in man when no such effects were found in test animals has drawn attention to the difficulties of screening substances for possible teratogenicity in man. Species, and even strain, differences often result in decidedly different responses to the same agent and this undoubtedly is related to genetic make-up.

Even where a drug is non-teratogenic for the majority of humans, there is always the possibility of teratogenic effect in a few individuals on account of their genetic constitution. This, however, is no different from drug sensitivity or post-vaccinal conditions which we are accustomed to anticipate in a small number of cases. New drugs, of course, must be intensively screened in a greater variety and number of test animals than before. This will help to reduce the chance of disaster in man. Also, we should not fail to check the old established drugs, the long-trusted components of the physician's armamentarium. In this regard, the demonstration of teratogenic action by salicylates[29] in rats should be kept in mind. In view of our present knowledge, avoiding drugs of all kinds in the early stages of pregnancy unless deemed absolutely necessary by the physician is obvious.

The quest for information on the causation of malformations also requires the detailed study of aborted human embryos. Too often normality or abnormality is determined by external inspection alone and, since a normal-looking embryo can have severe visceral abnormalities within, a diagnosis is of little value unless based on dissection, and possibly on histological and biochemical studies as well. Detailed examination of such material is time-consuming and requires special skills, but it must be undertaken and, wherever possible, the findings related to the maternal history. The establishment of centers to which human abortion material could be sent for special study would doubtless facilitate such an undertaking.

Lastly, while laboratory studies have an important part in the detection of teratogenic agents, an equally significant role is played by the practicing physician, for by astute observation and careful recording he can, as he has so often in the past, draw attention to actual or potential dangers and open the way to appropriate safeguards.

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